

# The Role of Plant Mediator as a Gene Regulator to Tackle Climate Change: A Review

D. L. Wathugala

Dept. of Crop Science, Faculty of Agriculture,  
University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka

Tele: (94) 71 826 5218; Fax: (94) 41 229 2384; E-mail: wgdlakmini@crop.ruh.ac.lk

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## ABSTRACT

*The current rate of climate change predicts that plants will become subject to increasing extremes of environmental stress. Rapid population increase in developing countries also demand higher yield from crop production, often from sub-optimal agricultural areas. Genetic engineering can help to meet these needs through the development of crops with greater stress tolerance. Mediator is transcriptional co-activators which convey DNA bound transcriptional regulators and enhancers to the general RNA polymerase II transcription machinery and mediator genes are recently identified in plants. So far it has been showed that their great involvement in regulation of plant stress tolerance by controlling transcription of stress genes. Therefore, Mediator genes will be a good source in future molecular biology researches on manipulation of crop plants with multiple stress tolerance.*

**KEYWORDS:** *Mediator genes, Environmental stress, Stress tolerance*

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## Introduction

Developing stress tolerant crops to cope with the rapid environmental degradation that is occurring is an absolute requirement in order to provide enough food for growing population. One of the basic genetic engineering approaches currently being used to improve crop stress tolerance is generation of transgenic plants by introducing novel genes into the genome of agriculturally important crops or altering the expression of existing genes. Understanding stress response signaling pathways is the prime requirement to manipulate stress tolerance of crop plants by this approach. Recent studies have made progress in the definition of various genetic protective responses to stress stimuli using the model plant species *Arabidopsis thaliana*. However, almost all these studies focused on only one stress condition. Therefore, finding ways to produce multiple stress tolerant crops are important. Mediator is the evolutionary conserved multi-protein complex that binds RNA polymerase II and controls transcription of genes (Flanagan *et al.*, 1991). The possible mechanism suggested by Chadick and Asturias (2005), Bjorklund and

Gustafsson (2005), and Hahn (2004) for transcriptional activation by mediator was that the gene specific activators (transcription factors) recruits mediator to the transcription initiation site. Then, general transcription factors involved in gene transcription interact with the DNA-mediator complex to form a platform to bind RNA polymerase II.

Therefore, the focus of this review is to provide information on the plant mediator to generate interest among researchers as a possible route to improve crop tolerance to environmental stresses. It first provides a short overview of past research that discusses changes of gene expression in response to environmental stresses. Next it describes transcription factors that regulate stress responses. The role of plant mediators in stress gene expression is then discussed. Then the paper concludes by summarizing the importance of future research needs of plant mediator as a possible route to improve crop tolerance to multiple environmental stresses.

### ***Changes in Gene Expression in Response to Environmental Stresses***

Stress responses are initiated when plants recognize stress at the cellular level, followed by generation of second messengers such as calcium and ROS (Reactive Oxygen Species). These then trigger activation of various signal transduction pathways (Fowler and Thomashow, 2002; Knight and Knight, 2001) which transmit information and activate stress inducible gene expression. Microarrays in different plant species show a significant overlap in the types of genes whose expression is altered upon exposure to different stresses (Schenk *et al.*, 2000; Chen *et al.*, 2002; Seki *et al.*, 2002). For example, Seki *et al.* (2002) report that 22 genes (out of 7000 from *Arabidopsis*) form part of a common transcriptional response to cold, drought and salt induced stresses.

Another microarray analysis, by Chen *et al.* (2002) using 402 *Arabidopsis* transcription factors, showed extensive overlaps in expression of similar genes under different stresses. Nawrath *et al.* (2002) also showed the induction of a number of genes in common between pathogen attack and UV irradiance. The encoded proteins function either to protect cells from stress damage directly, or are involved in the generation of regulatory molecules like abscisic acid (ABA) ethylene and salicylic acid (SA) which themselves further regulate gene expression (Shinozaki *et al.*, 2003). The signaling pathways of stress induced gene expression are not yet completely understood, though the transcriptional activation of some stress induced genes has been well studied (Xiong *et al.*, 2002).

### ***Transcription Factors Regulate Stress Responses***

Transcription factors are genetic switches which fine tunes all responses of plants by activating promoters of inducible genes. Many transcription factor genes are found among the list of genes up and down regulated in response to environmental

stresses (Seki *et al.*, 2001; Kreps *et al.*, 2002; Seki *et al.*, 2002; Chen *et al.*, 2002; Shinozaki *et al.*, 2003).

Stress-inducible expression of transcription factors include members of the AP2/EREBP (apetala2/ethylene-responsive element binding proteins) family, the zinc-finger family, the WRKY family<sup>1</sup>, the MYB (myeloblastosis) family, the basic helix–loop–helix (bHLH) family, the basic-domain leucine zipper (bZIP) family, the NAC family<sup>2</sup>, and the homeodomain transcription factor family (Shinozaki *et al.*, 2003). The AP2/EREBP transcription factor family, bZIP family and WRKY family proteins are reported as major players in the regulation of gene expression in response to environmental stresses (Singh *et al.*, 2002). Furthermore, significant improvement of stress tolerance was observed upon over-expression of individual transcription factors from these families. As examples, over-expression of *CBF1/DREB1B* and *DREB1A* (sub families of AP2/EREBP family) in *Arabidopsis* greatly increases the plant's resistance to cold, drought and salt (Jaglo-Ottosen *et al.*, 1998; Kasuga *et al.*, 1999).

Both induction and repression of specific transcription factors are required to fine tune the plant to various environmental stresses. For example, MYB transcription factors are associated with responses to UV light and pathogen infection (Rushton and Somssich, 1998; Singh *et al.*, 2002), though Jin and colleagues (2000) report that *AtMYB4* represses target genes under UV-B irradiance, and the over-expression of *AtMYB4* reduces the UV-B tolerance of *Arabidopsis*.

AP2/EREBP comprises the biggest transcription factor family in *Arabidopsis*, with 45 members (Riechmann *et al.*, 2000). These transcription factors are linked with a wide range of stress responses. One subfamily of AP2/EREBP, known as CBF/DREB1 (C-repeat binding factor/drought responsive element binding factor1), bind a *cis*-element known as the CRT/DRE (C-repeat/drought responsive element), common to the promoters of *COR* (Cold on regulated) genes, induced by cold, drought and salt-stress (Stockinger *et al.*, 1997; Liu *et al.*, 1998). In *Arabidopsis* there are four CBF/DREB1 proteins. Another DRE binding protein, DREB2 (drought responsive element binding factor2); is from a subfamily of AP2/EREBP transcription factors that regulate several drought and salt stress induced genes (Liu *et al.*, 1998). A further transcription factor subfamily, known as ERFs (ethylene responsive element binding factors), bind to GCC box *cis*-element in the promoters of several pathogenesis related (*PR*) genes (Singh *et al.*, 2002). Therefore, members of the AP2/EREBP family regulate both biotic and abiotic stress responsive gene expression.

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<sup>1</sup> The conserved amino acid sequence of WRKY domain is WRKYGQK at its N terminal end and the first letters of this sequence use to name WRKY domain.

<sup>2</sup> NAC domain consist three different genes such as NAM (no apical meristem), ATAF (Arabidopsis transcription activation factor) and CUC (cup-shaped cotyledon) and first letters of each gene use to name NAC domain.

The bZIP family of transcription factors also functions to control stress responsive gene expression. One type of bZIP family transcription factors, the ABFs (Abscisic acid binding factors) bind ABREs (Abscisic acid responsive elements). These cis-elements regulate expression of cold, drought and salt induced genes via ABA-dependent signal transduction (Choi *et al.*, 2000). Over-expression of ABF3 (Abscisic acid binding factor3) and ABF4 transcription factors in *Arabidopsis* alters the expression of stress responsive genes and increases drought tolerance in transgenic plants (Kang *et al.*, 2002). Another bZIP subfamily, the TGA (TGACG motif binding factor)/OBF (ocs element binding factor), bind to the *as-1/ocs* element of *PR1* genes (Zhang *et al.*, 1999; Despres *et al.*, 2000). NPR1 (non expresser of PR1), a key component of SA (Salicylic Acid) mediated signaling, interacts with TGA/OBF family proteins upon pathogen infection. This physical interaction is essential to enhance the DNA-binding properties of TGA/OBFs which lead to the induction of *PR* gene transcripts (Eulgem, 2005).

WRKY transcription factors are unique to plants, and are involved in stress responsive gene expression. Members of the WRKY family show enhanced expression following pathogen infection (Singh *et al.*, 2002). As an example, the expression of WRKYs is essential to activate *NPR1* activity upon pathogen infection (Yu *et al.*, 2001). WRKY factors interact with two W boxes in the *NPR1* 5' UTR upon pathogen infection, activating *NPR1* transcription. Over-expression of several *WRKY* genes in *Arabidopsis* has produced an enhanced resistance to pathogens and altered expression of several stress responsive genes (Singh *et al.*, 2002).

The MYB and bHLH families of transcription factors are also reported to be involved in stress-responsive gene expression. These transcription factors specifically bind to MYC and MYB recognition sites in promoters of ABA responsive genes (Abe *et al.*, 2003). Transgenic *Arabidopsis* over-expressing *AtMYC2* and *AtMYB2* transcription factors genes showed a higher sensitivity to ABA and altered expression of ABA induced genes (Abe *et al.*, 2003).

The above analysis of current literature shows that, many transcription factors are involved in regulation of stress responsive gene expression. Some of the stress genes are induced by several transcription factors, whilst the same transcription factor may behave differently in response to different stress stimuli. Therefore, it is important to understand the mechanism by which transcription factors are recruited to the promoters of stress induced genes, in order to manipulate the stress resistance of crop plants.

### ***General RNA Polymerase II Transcription Cycle***

The process of synthesis of RNA from DNA is called transcription. This process takes place in the nucleus. The cell initiates transcription by directing the RNA

polymerase II complex to motifs in the DNA in proximity to the coding region of the target gene, known as the promoter. The most common promoter motif in eukaryotic DNA is the TATA box. Transcription initiates through the formation of a pre-initiation complex (PIC) near the transcriptional start. The PIC includes 6 general transcription factors (GTFs) and the RNA polymerase II enzyme (RNAPII). These GTFs are known as TFIIA, TFIIB, TFIID, TFIIE, TFIIIF, TFIIF (Hahn, 2004; Thomas and Chiang, 2006); they work together with RNAPII to specify the transcription initiation site (Thomas and Chiang, 2006).

The PIC usually begins with TFIID binding to the TATA box, followed by the entry of the other GTFs and RNAPII either in a sequential assembly or a preassembled RNAPII holoenzyme (Thomas and Chiang, 2006). Formation of a PIC is sufficient for basal level transcription (Thomas and Chiang, 2006). However, for activator-dependent (or regulated) transcription, general co-factors are also required. These include mediator, TAFs (TATA Binding Protein associated factors) and upstream stimulatory activity (USA) derived positive and negative co-factors, and are needed to transmit regulatory signals between gene specific activators and the general transcription machinery (Thomas and Chiang, 2006).

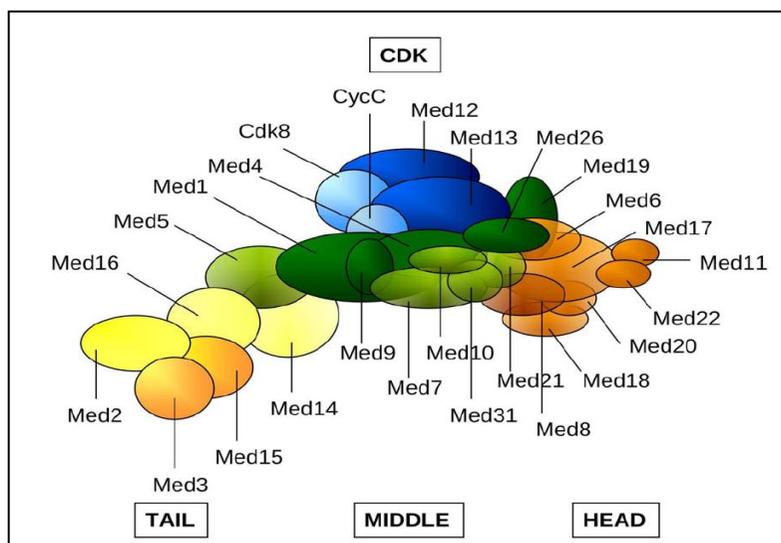
With the completion of formation of PIC, dramatic conformational changes occur in the 11-15 base pairs of DNA surrounding the transcription start (Hahn, 2004). Then transcription begins with the synthesis of the first phosphodiester bond of RNA (Hahn, 2004). In several transcription systems, multiple short RNAs, called abortive products, are synthesized before initiating synthesis of full length transcripts (Hahn, 2004).

After synthesis of about 30 bases of RNA, RNAPII, TFIIB, and TFIIIF release their contact with the core promoter and the rest of the transcription machinery enter the transcript elongation phase (Hahn, 2004). Many of the GTFs of PIC remain behind at the promoter in the scaffold complex after release of RNAPII from the PIC complex, and this enables re-initiation of transcription (Hahn, 2004). The scaffold complex accelerates subsequent transcription by bypassing the very slow recruitment of GTFs and formation of PIC at the transcriptional start site (Hahn, 2004).

### ***Mediator Complex***

Mediator is a transcriptional co-activator complex which acts as a bridge to convey DNA-bound transcriptional regulators and enhancers to the general RNA polymerase II transcription machinery (Chadick and Asturias, 2005; Bourbon, 2008). Components of mediator were first identified through biochemical and genetic studies in baker's yeast (*Saccharomyces cerevisiae*), as subunits linking to the RNA Polymerase II holoenzyme (Flanagan *et al*, 1991). Mediator complexes were subsequently found in other organisms including higher eukaryotes (Bourbon *et al.*, 2004; Bjorklund and Gustafsson, 2005; Bourbon, 2008).

Of these, the structure and composition of yeast mediator has been studied in most detail. The yeast complex comprises 26 subunits (Guglielmi *et al.*, 2004), and has been grouped through interactor studies into 4 sub-modules known as the head, middle, tail and kinase (CDK) (Asturias *et al.*, 1999) (Figure 1). In mammalian mediator, 30 subunits have been identified (Bourbon *et al.*, 2004). However, prior to 2007 there were no reports of a plant mediator complex, probably due to the low sequence homology between *Arabidopsis* and other non-plant eukaryotic mediators (Backstrom *et al.*, 2007).



Source: Guglielmi *et al.* (2004)

**Figure 1: Topological organization of yeast mediator**

Physical interactions between mediator subunits were revealed in 1992 by Jiang and Stillman, through research on yeast SIN4 (MED16) subunit mutant strain. A mutation in the SIN4 (MED16) subunit of yeast showed loss of function of other tail subunits and therefore, it was suggested that mediator subunits physically interact with each other (Jiang and Stillman, 1992). Consistent with these results, electron microscopic (EM) studies of holoenzyme complexes purified from mutant yeast lacking MED16, showed that the tail mediator modules (MED15, MED3 and MED2) were absent (Chadick and Asturias, 2005). Therefore, they also suggested that the tail portion of mediator formed a subset of physically interacting units. Moreover, the physical association of yeast MED16 and MED14 (Rgr1) proteins were also apparent with the same spectrum of phenotypes observed in *sin4* and *rgr1* mutant strains (Jiang *et al.*, 1995).

## Mediator in Transcriptional Regulation

During transcriptional activation and repression, the mediator complex is not directly in contact with gene-specific regulatory sequences (enhancers or silencers). Rather, mediator subunits bind to specific transcriptional activators or repressors, and with other components of PIC (Figure 2) (Bjorklund and Gustafsson, 2005; Bourbon, 2008). Studies have shown that yeast mediator is present both free and in a complex with RNAPII (mediator+RNAPII) (Kim *et al.*, 1994). However, whether the mediator recruit either as free complex or mediator+RNAPII complex to PIC to initiate transcription is not well understood yet.

The CDK module (Srb8-11 or according to the new nomenclature MED12, MED13, Cdk8 and CycC respectively) of mediator has been identified as a negative regulator of transcription. Therefore, mediator complexes with the CDK module prevent interaction with RNAPII. Degradation of the CDK module is necessary for mediator to interact with RNAPII and subsequently initiate transcription (Samuelson *et al.*, 2003) (Figure 2). These same authors proposed a three step model for the degradation of CDK before transcription can commence. First, mediator complexed with CDK binds the transcriptional activation domain, and then to the promoter of the gene; finally at the promoter, the CDK module degrades to facilitate interaction with RNAPII. This means that the mediator attached to PIC comprises only the head, middle and tail modules. Consistent with these results, EM visualization of yeast mediator attached to PIC did not revealed the CDK module (Chadick and Asturias, 2005).

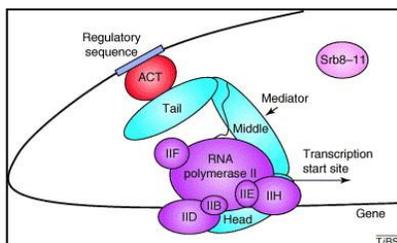


Figure 2a. Transcription activation

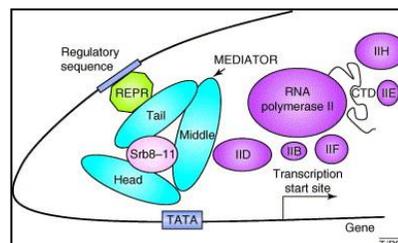


Figure 2b. Transcription repression

**Figure 2: A. Mediator functions as a bridge between gene-specific activators and the general polymerase II transcription machinery at the promoter. Activator interactions mainly take place within the tail region of mediator, whereas contacts with polymerase II are localized to the head and middle region. B. Gene specific repressors (REPR) interact with specific mediator subunits and recruit the complex to upstream regulatory DNA sequences. Mediator that is recruited by repressors contains the Srb8-11 (Med12-Med13-Cdk8-CycC) module, which prevents interactions with polymerase II and the basal transcription machinery (Source: Bjorklund and Gustafsson, 2005)**

EM images of yeast PIC revealed that the tail domain extends away from RNAPII, though there are multiple physical contacts between RNAPII and the middle and head mediator domains (Chadick and Asturias, 2005). However, large areas of the RNAPII surface (~ 75%) remain available for interaction with other components of the PIC (Chadick and Asturias, 2005). When considering the structural changes of mediator complex during RNAPII interaction, it appears that the structure of the head domain remains same but the tail and middle domains unfold (Chadick and Asturias, 2005).

The carboxyl terminal domain (CTD) of Rpb1 (the largest RNAPII subunit) was identified as necessary for regulation of transcription through mediator (Myers *et al.*, 1998; Chadick and Asturias, 2005). However, more than CTD is required to facilitate the conformational changes necessary for the interaction of mediator with RNAPII (Chadick and Asturias, 2005). In addition, the mediator-RNAPII interaction appears to be at least kingdom-specific, since no mediator+RNAPII complex was formed when yeast mediator was incubated with mammalian RNAPII. This is surprising since the sequences of yeast and mammalian CTDs are highly homologous (Chadick and Asturias, 2005).

### ***Plant Mediator***

In 2007, Backstrom *et al.* were able to identify plant mediator complexes from *Arabidopsis thaliana*, using a combination of bioinformatics and biochemical analytical techniques. They found that most of the yeast and metazoan subunits are present in plant mediator, but with a very low sequence homology. Since the *Arabidopsis* genome encodes ~700 plant specific transcription factors, to regulate these via mediator, the plant protein recognition motifs would have to differ from those of yeast and other eukaryotes subunits (Backstrom *et al.*, 2007). Their study found homologues for most head and middle domain subunits known from yeast, but very low homology for the candidates of tail domain.

This section of the mediator complex also showed greatest variability when comparing proteins from yeast and metazoans (Backstrom *et al.*, 2007). As mentioned earlier, tail domains make contact with gene-specific enhancers and suppressors of transcriptional regulation. It is likely that the *Arabidopsis* mediator includes plant specific tail domains (Backstrom *et al.*, 2007). Apart from that they identified potential plant specific mediator subunits named as MED32-37. Backstrom *et al.* (2007) also identified *PFT1* (*phytochrome and flowering time*; Cerdan and Chory, 2003) as *MED25* and *SWP* (*struwwelpeter*; Autran *et al.*, 2002) as *MED14*. These genes were already known as being involved in regulation of flowering time via phyB-dependent pathway (*PTF1*), and defining the duration of cell proliferation (*SWP*).

Recently Kidd *et al.* (2009) showed that *PFT1* is a key regulator of jasmonate dependent defense in *Arabidopsis* which is an important defense pathway activate

upon pathogen infection. Very recently, it has been observed the great involvement of MED16 on various pathways of stress tolerant gene expression such as CBF gene expression on freezing and osmotic stresses and UV and pathogen response gene expression (Knight *et al.*, 2009; Wathugala *et al.*, 2012). However, overexpression of MED16 in *Arabidopsis* did not increase cold gene expression indicating levels of MED16 alone might not influence activation of target genes (Wathugala *et al.*, 2011). The orthologue of *Arabidopsis* MED16 has also been identified in rice (Wathugala *et al.*, 2011) and homologues of *Arabidopsis* MED16 are presented in many agricultural crop species such as maize, papaya, rye *etc.*

## Conclusions

It is clear that mediator subunits combine in a complex in equal molar ratios. Moreover, mediator subunits are linked to each other and these links are essential to get maximum output of mediator function on regulation of transcription. Therefore, it can be suggested that the over-expression of one subunit of the mediator complex might not affect the function of the complex as a whole. Therefore, over-expression of one subunit of mediator will not be able to be used as a basic molecular tool to improve crop tolerance to stress. However, engineering the protein sequences to improve efficiency and identification of transcription binding sites in mediator subunits will be able to optimize transcription factor binding and it might lead to improve plant tolerance to environmental stresses. Also, before manipulation of protein sequences of mediator complex proper understanding of the function of mediator subunits on regulation of stress induced gene expression is a must. Therefore, future research on specific roles of individual subunits and of the whole complex will widen our knowledge of the transcriptional regulation of gene expression in plant and will create new routes to improve crop tolerance to multiple environmental stresses.

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